

CLAIMS

1. Process for the preparation of an epiK5-N,O-oversulfate-derivative, characterized in that

(a) an epiK5-N-sulfate-derivative having a mean molecular weight of between approximately 1,500 and approximately 25,000, in acidic form, is treated with tertiary or quaternary organic base, letting the reaction mixture to stand for a time period of 30-60 minutes at a pH of approximately 7 and its salt is isolated with said organic base;

(b) said salt of organic base of said epiK5-N-sulfate-derivative is treated with an O-sulfation reagent in the conditions of O-oversulfation;

(c) a salt of tertiary or quaternary organic base of epiK5-amine-O-oversulfate-derivative thus obtained is treated with a reagent of N-sulfation and the epiK5-N,O-oversulfate-derivative thus obtained is isolated.

2. Process according to claim 1, characterized in that said epiK5-N,O-oversulfate-derivative is isolated in sodium salt form and optionally transformed into another chemically or pharmaceutically acceptable salt.

3. Process according to anyone of claims 1 and 2, characterized in that, in step (a) tetrabutylammonium hydroxide is used as an organic base.

4. Process according to anyone of claims from 1 to 3, characterized in that, in step (b) the O-oversulfation is carried out in dimethylformamide using 2-4 moles of O-sulfation reagent per available OH per disaccharide at a temperature of 40-60°C for 15-20 hours.

5. Process according to anyone of claims from 1 to 4, characterized in that an epiK5-N-sulfate-derivative is used as starting material having a

mean molecular weight from approximately 1,000 to approximately 25,000.

6. Process according to claim 5, characterized in that said starting epiK5-N-sulfate-derivative is 40-60% C5-epimerized.

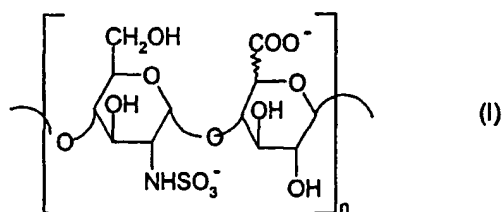
7. Process according to anyone of claims from 1 to 6, characterized in that said starting epiK5-N-sulfate-derivative has a mean molecular weight from approximately 1,500 to approximately 25,000.

8. Process according to claim 7, characterized in that said starting epiK5-N-sulfate-derivative has a mean molecular weight between 10,000 and 25,000.

9. Process according to anyone of claims from 1 to 6, characterized in that said starting material has a mean molecular weight from approximately 1,000 to approximately 12,000.

10. Process according to claim 9, characterized in that said starting material has a mean molecular weight from approximately 1,500 to approximately 8,000.

11. Process according to claim 5, characterized in that an epiK5-N-sulfate-derivative is used as starting material consisting of a chain mixture in which at least 90% of said chains have the formula I



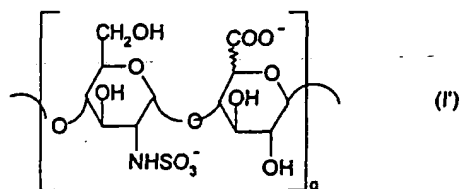
in which the uronic units are 20-60% consisting of iduronic acid, n is a integer from 2 to 100 and the corresponding cation is chemically or pharmaceutically acceptable.

12. Process according to claim 11, characterized in that said starting material consists of a chain mixture in which at least 90% of said chains

have the formula I, in which the uronic units are 40-60% consisting of iduronic acid.

13. Process according to anyone of claims 11 and 12, characterized in that, in the formula I, n represents a integer from 3 to 100.

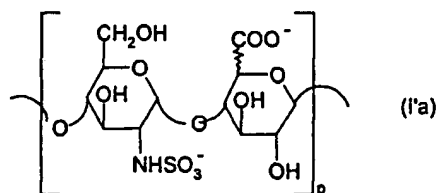
14. Process according to claim 5, characterized in that said starting material consists of a chain mixture in which at least 90% of said chains have the formula I'



in which the uronic units are 20-60% consisting of iduronic acid, q is a integer from 2 to 20 and the corresponding cation is chemically or pharmaceutically acceptable.

15. Process according to claim 14, characterized in that said starting material consists of a chain mixture in which at least 90% of said chains have the formula I', in which n is a integer from 3 to 15.

16. Process according to claim 5, characterized in that said starting material consists of a chain mixture in which the preponderant species has the formula I'a

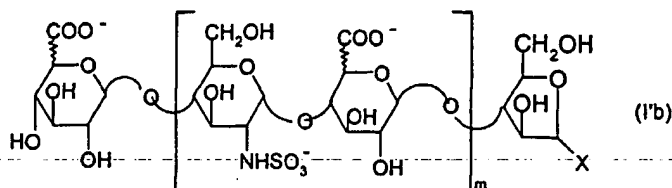


in which the uronic units are 60-40% consisting of glucuronic acid and 40% to 60% of iduronic acid, p is a integer from 4 to 8 and the corresponding cation is chemically or pharmaceutically acceptable.

17. Process according to claim 16, characterized in that the mean

molecular weight of said starting material is from approximately 2000 to approximately 4000.

18. Process according to anyone of claims 16 and 17, characterized in that said starting material consists of a chain mixture in which the preponderant species has the formula I'b



in which X is hydroxymethyl, m is 4, 5 or 6 and the glucuronic and iduronic units are present alternately, starting with a glucuronic or iduronic unit.

19. Process according to anyone of claims from 5 to 18, characterized in that said starting material comes from an N-deacetylation and from an N-sulfation of a K5 that is basically free of lipophilic substances.

20. An epiK5-N,O-oversulfate-derivative obtainable according to the process of claims from 1 to 19.

21. An epiK5-N,O-oversulfate-derivative having an iduronic acid content of 20-60%, a mean molecular weight from approximately 2,000 to approximately 45,000 and a sulfation degree of at least 4, or one of its chemically or pharmaceutically acceptable salts, said derivative being basically inactive on the coagulation parameters.

22. An epiK5-N,O-oversulfate-derivative according to claim 21, whose mean molecular weight is between approximately 15,000 and approximately 45,000.

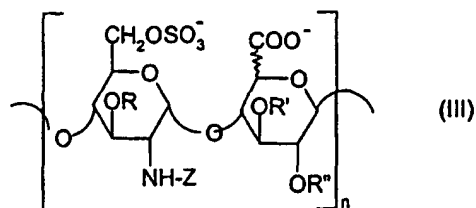
23. An epiK5-N,O-oversulfate-derivative according to claim 21, whose mean molecular weight is between approximately 4,500 and approximately 8,500.

24. An epiK5-N,O-oversulfate-derivative according to anyone of

claims from 21 to 23, characterized in that said degree of sulfation is from 4 to 4.6.

25. An epiK5-N,O-oversulfate-derivative according to anyone of claims from 21 to 24, characterized in that it is 100% 6-O-sulfated and 50-80% 3-O-sulfated in its glucosamine units, 5-10% O-monosulfated in glucuronic units, 10-15% 3-O-monosulfated in iduronic units and 2,3-di-O-sulfated in the remaining uronic units.

26. An epiK5-N,O-oversulfate-derivative according to anyone of claims from 21 to 25, characterized in that it consists of a chain mixture in which at least 90% of said chains have the formula III



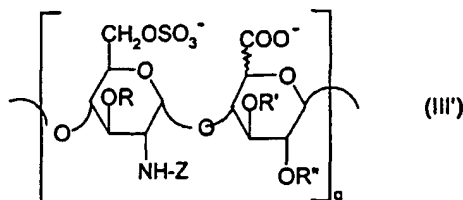
in which the uronic units are 20-60% consisting of iduronic acid, R, R', R'' represent hydrogen or SO₃⁻, R being SO₃⁻ in at least 40% of said chain mixture, Z is an SO₃⁻ group, n is a integer from 2 to 100, the degree of sulfation is at least 4 and the corresponding cation is chemically or pharmaceutically acceptable.

27. An epiK5-N,O-oversulfate-derivative according to claim 26, characterized in that it consists of a chain mixture in which at least 90% of said chains have the formula III, in which the uronic units are 40-60% iduronic acid.

28. An epiK5-N,O-oversulfate-derivative according to anyone of claims 26 and 27, characterized in that it consists of a chain mixture in which at least 90% of said chains have the formula III, in which n is a integer from 3 to 100.

29. An epiK5-N,O-oversulfate-derivative according to anyone of

claims from 26 to 28, characterized in that it is a LMW-epiK5-N,O-oversulfate consisting of a chain mixture in which at least 90% of said chains have the formula III'



in which the uronic units are 20-60% consisting of iduronic acid, q is an integer from 2 to 20, R , R' and R'' represent hydrogen or an SO_3^- group, Z is SO_3^- , for a sulfation degree of at least 4, from 4 to 4.6, and the corresponding cation is one chemically or pharmaceutically acceptable ion.

30. A LMW-epiK5-N,O-oversulfate according to claim 29, characterized in that it consists of a chain mixture in which at least 90% of said chains have the formula III' in which q is an integer from 3 to 15.

31. A LMW-epiK5-N,O-oversulfate according to claim 30, characterized in that it consists of a chain mixture in which at least 90% of said chains have the formula III' in which the uronic units are 40-60% consisting of iduronic acid.

32. A LMW-epiK5-N,O-oversulfate according to claim 31 characterized in that its iduronic acid content is 50-55%.

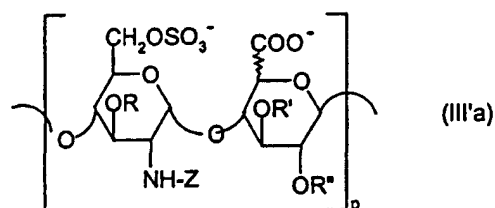
33. A LMW-epiK5-N,O-oversulfate according to anyone of claims from 29 to 32, characterized in that it consists of a chain mixture in which at least 90% of said chains have the formula III' in which R is at least 40% SO_3^- , R' and R'' are both SO_3^- or one is hydrogen and the other is 5-10% SO_3^- in glucuronic acid and 10-15% SO_3^- in iduronic acid.

34. A LMW-epiK5-N,O-oversulfate according to claim 33 characterized in that it has a mean molecular weight from approximately 2,000 to approximately 16,000.

35. A LMW-epiK5-N,O-oversulfate according to claim 34 characterized in that it has a molecular weight from approximately 4,500 to approximately 9,000.

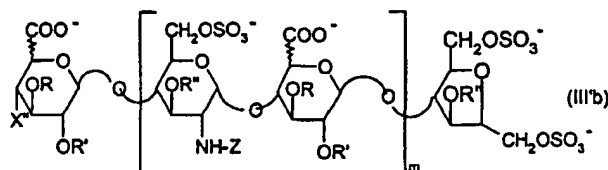
36. A LMW-epiK5-N,O-oversulfate according to anyone of claims from 33 to 35, characterized in that it consists of a chain mixture in which at least 90% of said chains have the formula III' in which R is 50-80% SO_3^- .

37. A LMW-epiK5-N,O-oversulfate according to anyone of claims from 32 to 36, characterized in that it consists of a chain mixture in which the preponderant species has the formula III'a



in which the uronic units are 20-60% consisting of iduronic acid, p is a integer from 4 to 8, Z is SO_3^- , R, R' and R'' are hydrogen or SO_3^- , for a degree of sulfation from 4 to 4.6 and the corresponding cation is chemically or pharmaceutically acceptable.

38. A LMW-epiK5-N,O-oversulfate according to anyone of claims from 33 to 37, characterized in that it consists of a chain mixture in which the preponderant species has the formula III'b



in which R, R' and R'' are hydrogen or SO_3^- , Z is SO_3^- , X'' is OH or OSO_3^- , m is 4, 5 or 6, for a degree of sulfation from 4 to 4.6, the glucuronic and iduronic units are present alternately, starting with a glucuronic or iduronic unit, and the corresponding cation is one chemically or pharmaceutically acceptable ion.

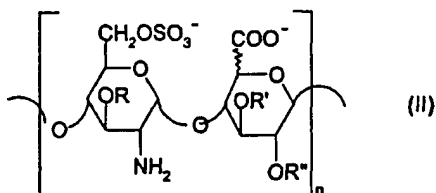
39. An epiK5-N,O-oversulfate-derivative according to anyone of claims from 20 to 38, characterized in that said chemically or pharmaceutically acceptable salt or cation is the salt or cation of an alkaline metal, alkaline-earth metal, ammonium, (C₁-C₄)tetraalkylammonium, aluminum and zinc.

40. An epiK5-N,O-oversulfate-derivative according to claim 39, characterized in that said chemically or pharmaceutically acceptable salt or cation is the salt or cation of sodium, calcium or tetrabutylammonium.

41. An epiK5-amine-O-oversulfate-derivative, one of its chemically or pharmaceutically acceptable salts, obtainable according to steps (a) and (b) of the claim 1, isolated in sodium salt form and, optionally, transformed into another chemically or pharmaceutically acceptable salt.

42. An epiK5-amine-O-oversulfate-derivative whose iduronic acid content is 20-60% of the total of the uronic acids, having a mean molecular weight from approximately 2,000 to approximately 40,000 and a sulfation degree of at least 3.4, or one of its chemically or pharmaceutically acceptable salts.

43. An epiK5-amine-O-oversulfate-derivative according to claim 42, characterized in that said epiK5-amine-O-oversulfate-derivative consists of a chain mixture in which at least 90% of said chains have the formula II



in which the uronic units are 20-60% consisting of iduronic acid, n is a integer from 2 to 100, R, R' and R'' are hydrogen or SO₃⁻, the degree of

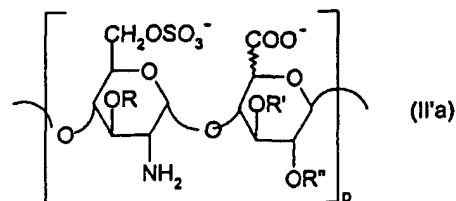
sulfation is at least 3.4 and the corresponding cation is chemically or pharmaceutically acceptable.

44. An epiK5-amine-O-oversulfated-derivative according to claim 43, characterized in that, in the formula II, n represents a integer from 3 to 100.

45. An epiK5-amine-O- oversulfated-derivative according to anyone of claims from 42 to 44, characterized in that it consists of a chain mixture in which at least 90% of said chains have the formula II in which the uronic units are 40-60% consisting of iduronic acid, with a mean molecular weight from approximately 2,000 to approximately 40,000, R is at least 40% SO_3^- , R' and R'' are both SO_3^- or one is hydrogen and the other is 5-10% SO_3^- in monosulfate glucuronic acid and 10-15% SO_3^- in monosulfate iduronic acid.

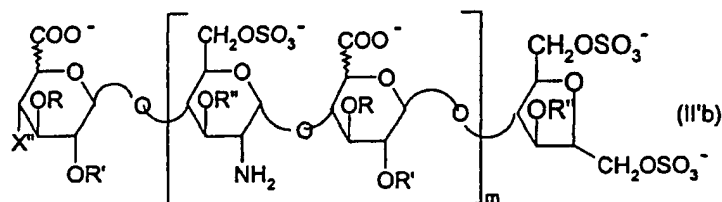
46. An epiK5-amine-O-oversulfate-derivative according to anyone of claims from 42 to 45, characterized in that it is a LMW-epiK5-amine-O-oversulfate consisting of a chain mixture in which at least 90% of said chains have the formula II in which the uronic units are 40-60% consisting of iduronic acid, R is at least 40% SO_3^- , R' and R'' are both SO_3^- or one is hydrogen and the other is 5-10% SO_3^- in glucuronic acid and 10-15% SO_3^- in iduronic acid, n is a integer from 3 to 15, with a mean molecular weight from approximately 4,000 to approximately 8,000 and the corresponding cation is chemically or pharmaceutically acceptable.

47. An epiK5-amine-O-oversulfate-derivative according to anyone of claims from 42 to 46, characterized in that it is a LMWepiK5-amine-O-oversulfate consisting of a chain mixture in which the preponderant species has the formula II'a



in which the uronic units are 20-60% consisting of iduronic acid, p is a integer from 4 to 8, R , R' and R'' are hydrogen or SO_3^- , the degree of sulfation is at least 3.4.

48. A LMW-epiK5-amine-O-oversulfate according to claim 47 characterized in that it consists of a chain mixture in which the preponderant species is a compound of formula II'b



in which the uronic units are 40-60% consisting of iduronic acid, m is 4, 5 or 6, R , R' and R'' are hydrogen or SO_3^- , X'' is OH or OSO_3^- , for a sulfation degree of at least 3.4, the iduronic units being present alternately, starting with a glucuronic or iduronic unit.

49. A LMW-epiK5-N-sulfate virtually free of NH_2 and N-acetyl groups, having an iduronic acid content from 20 to 60% and a mean molecular weight from approximately 1,500 to approximately 12,000, or one of its chemically or pharmaceutically acceptable salts.

50. A LMW-epiK5-N-sulfate according to claim 49, characterized in that the iduronic acid content is from 40 to 60% and the mean molecular weight is from approximately 1,500 to approximately 10,000.

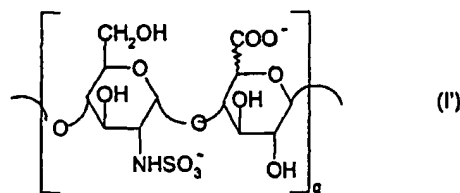
51. A LMW-epiK5-N-sulfate according to claim 49, characterized in that the iduronic acid content is 50-55% and the mean molecular weight is from approximately 1,500 to approximately 7,500.

52. A LMW-epiK5-N-sulfate according to anyone of claims from 49 to 51, obtained by a process characterized in that a K5-N-sulfate is subjected, in any one order,

- (i) to C5-epimerization with a D-glucuronyl C5-epimerase isolated, purified and in solution or immobilized on a solid support, at a pH of approximately 7, at a temperature of approximately 30°C and for a time period of 12-24 hours in the presence of at least one bivalent ion selected among calcium, magnesium, barium and manganese; and
- (ii) to nitrous depolymerization optionally followed by reduction.

53. A LMW-epiK5-N-sulfate according to claim 52, characterized in that, in step (ii), the depolymerization is followed by reduction with sodium borohydride.

54. A LMW-epiK5-N-sulfate according to claim 53, characterized in that it consists of a chain mixture in which at least 90% of said chains have the formula I'



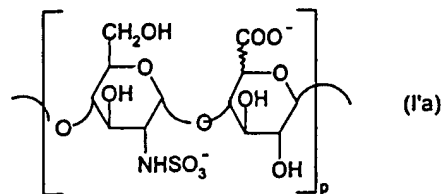
in which the uronic units are 20-60% consisting of iduronic acid, q is a integer from 2 to 20 and the corresponding cation is chemically or pharmaceutically acceptable.

55. A LMW-epiK5-N-sulfate according to claim 54, characterized in that it consists of a chain mixture in which at least 90% of said chains have the formula I', in which the uronic units are 40-60% iduronic acid.

56. A LMW-epiK5-N-sulfate according to anyone of claims 54 and 55, characterized in that it consists of a chain mixture in which at least 90% of said chains have the formula I', in which n is a integer from 3 to 15.

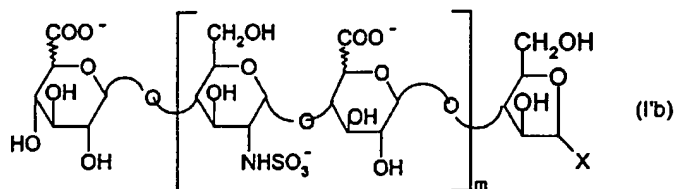
57. A LMW-epiK5-N-sulfate according to anyone of claims 54 and 55,

characterized in that it consists of a chain mixture in which the preponderant species has the formula I'a



in which the uronic units are 60-40% consisting of glucuronic acid and 40% to 60% iduronic acid, p is a integer from 4 to 8 and the corresponding cation is chemically or pharmaceutically acceptable.

58. A LMW-epiK5-N-sulfate according to anyone of claims from 55 to 57, characterized in that it consists of a chain mixture in which the preponderant species has the formula I'b



in which X is hydroxymethyl, m is 4, 5 or 6, the corresponding cation is one chemically or pharmaceutically acceptable ion and the glucuronic and iduronic units are present alternately, starting with a glucuronic or iduronic unit.

59. A LMW-epiK5-N-sulfate according to anyone of claims from 49 to 58, characterized in that said salt or cation is selected among those of alkaline metals, alkaline-earth metals, ammonium, (C₁-C₄)tetraalkylammonium, aluminum and zinc.

60. A LMW-epiK5-N-sulfate according to claim 59, characterized in that said salt or cation is selected among those of sodium, calcium and tetrabutylammonium.

61. Process for the preparation of a LMW-epiK5-N-sulfate,

characterized in that a K5-N-sulfate is subjected, in any one order,
(i) to C5-epimerization with a D-glucuronyl C5-epimerase isolated, purified and in solution or immobilized on a solid support, at a pH of approximately 7, at a temperature of approximately 30°C and for a time period of 12-24 hours in the presence of at least one bivalent ion selected among calcium, magnesium, barium and manganese; and
(ii) to nitrous depolymerization optionally followed by reduction.

62. Process according to claim 61, characterized in that it is carried out in the order (i)-(ii).

63. Process according to claim 61, characterized in that it is carried out in the order (ii)-(i).

64. Process according to claim 63, characterized in that the product obtained upon termination of the depolymerization is a LMW-K5-N-sulfate which is directly subjected to C5-epimerization.

65. Process according to claim 64, characterized in that said LMW-K5-N-sulfate has a mean molecular weight of more than 4,000.

66. A pharmaceutical composition including, as one of its active ingredients, a pharmacologically active amount of an epiK5-N,O-oversulfate-derivative according to anyone of claims from 20 to 40 in mixture with a pharmaceutical excipient.

67. A cosmetic composition including an effective amount of an epiK5-N,O-oversulfate-derivative according to anyone of claims from 20 to 40, in mixture with a cosmetic excipient.

68. Use of the isolated and purified C5-epimerase for the conversion of a K5-N-sulfate-derivative into a corresponding epiK5-N-sulfate-derivative characterized by a repetitive tetrasaccharide unit consisting of two glucosamine units separated by a glucuronic unit and followed by an iduronic unit or separated by an iduronic unit and followed by a glucuronic unit.

69. Use according to claim 68, characterized in that said K5-N-sulfate-derivative has a mean molecular weight of more than 4,000.

70. Use according to claim 69, characterized in that said mean molecular weight is from 6,000 to 7,500.